Chromosomal rearrangements in *Salmonella* spp. S2-2

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Abstrak

Studi genetik awal memperlihatkan adanya konservasi informasi gen dalam bakteri enterik. Dua metode mutakhir menggunakan PFGE untuk menentukan peta fisik genom adalah: (1) Digesti parsial dengan endonuklease I-CeuI yang memotong DNA bakteri di rrn operon dari rRNA (RNA ribosom), untuk penentuan "kerangka genomik rrn" (ukuran dalam kb jarak antara operon-operon rRNA). (2) Analisis situs XbaI dan BlnI dalam insersi Tn10 di kromosom. Urutan fragmen I-CeuI yang adalah ABCDEFG dalam S. typhimurium LT2 *dan* E. coli K-12, *ditemukan terkonservasi pada spesies salmonella yang sebagian besar tumbuh pada berbagai pejamu (pejamu umum). Akan tetapi pada spesies* S. typhi, S. paratyphi C, S. gallinarium *dan* S. pullorum *yang mempunyai pejamu tertentu, fragmen tersebut berbeda susunan karena rekombinasi homolog antara operon rrn menghasilkan translokasi dan inversi. 127 galur* S. typhi *tipe liar mempunyai 21 urutan berbeda dari fragmen I-CeuI. Inversi dan translokasi yang tidak melibatkan operon rrn jarang terdeteksi kecuali untuk inversi pada daerah TER (*Termination of replication*). Perubahan penambahan genetik (akibat transfer lateral yang menghasilkan insersi DNA tidak homolog) menghasilkan* Pathogenicity islands *yang berisi blok DNA yang memasukkan gen baru ke galur tertentu, sehingga mendorong terjadinya evolusi cepat dari sifat-sifat baru.*

Abstract

*Early genetic studies showed conservation of gene order in the enteric bacteria. Two recent methods using pulsed-field gel electrophoresis (PFGE) to determine the physical map of the genome are: (1) partial digestion with the endonuclease I-*CeuI*, which digests the DNA of bacteria in the* rrn *operon for rRNA (ribosomal RNA), thus establishing the "*rrn *genomic skeleton" (the size in kb of the intervals between rRNA operons); (2) analysis of* XbaI *and* BlnI *sites within Tn10 insertions in the chromosome. The order of I-*CeuI *fragments, which is ABCDEFG in* S. typhimurium *LT2 and* E. coli *K-12, was found to be conserved in most* Salmonella *species, most of which grow in many hosts (host-generalists). However, in* S. typhi, S. paratyphi C, S. gallinarum, *and* S. pullorum, *species which are host-specialized, these fragments are rearranged, due to homologous recombination between the* rrn *operons resulting in translocations and inversions. Inversions and translocations not involving the* rrn *operons are seldom detected except for inversions over the TER region (termination of replication). Additive genetic changes (due to lateral transfer resulting in insertion of non-homologous DNA) have resulted in "pathogenicity islands" containing blocks of DNA which provide new genes to specific strains, thus driving rapid evolution of new traits.*

Introduction

Gene orders in the chromosomes of enteric bacteria such as *Escherichia coli* K-12 and *Salmonella typhimurium* LT2 are reported to be strongly conserved[1,2]; the chromosomes of other genera such as *Bacillus* and *Streptomyces* are relatively unstable[3,4]. This extreme conservation in enteric bacteria is surprising, because during growth in culture, and presumably in nature, chromosomes frequently rearrange; duplications of segments of the chromosome occur at high frequencies (10^{-3} to 10^{-5})[5-7], and some inversions and translocations, especially those with endpoints in the *rrn* operons, are common[5,8]. The observed stability of the chromosome during evolution, in spite of the high frequency of rearrangements in culture, indicates that major forces must select against cells with rearrangements, resulting in elimination of cells in which the genome has rearranged.

The purposes of this review are to evaluate our present knowledge about the degree of stability of the genome of enteric bacteria, and to discuss the mechanisms which have contributed to bacterial evolution leading to virulence, especially in the genus *Salmonella*.

Methods of Genome Analysis

Mapping by genetic methods such as conjugation and transduction, used to construct linkage maps of *E. coli* K-12 and *S. typhimurium* LT2, is relatively slow, but physical methods using endonuclease digestion and PFGE permit rapid construction of genomic maps[3,9]. Two specific modifications of these methods have been used recently in our laboratory.

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I-*Ceu*I analysis

The endonuclease I-*Ceu*I together with PFGE is especially suitable for construction of a genomic cleavage map showing the number and locations of the *rrn* genes for rRNA (the "*rrn* skeleton")[10-12]. I-*Ceu*I is encoded by a class I mobile intron which is inserted into the *rrl* gene coding for the large subunit rRNA (23S-rRNA) in the chloroplast DNA of *Chlamydomonas eugamatos*[13]. I-*Ceu*I is specific for and cuts in a 19 bp sequence in the *rrl* gene[14]. Because rDNA sequences are strongly conserved, the I-*Ceu*I site is present in all seven *rrl* genes of enteric bacteria, but at no other site so far detected[10]. The order of I-*Ceu*I fragments and their approximate sizes for *S. typhimurium* LT2 are in Figure 1A; the order is the same in *E. coli* K-12 and the sizes are similar[15,16]. In addition, fragments that are adjacent on the chromosome can be determined by partial I-*Ceu*I digestion[11]. Preparation of high-molecular weight genomic DNA, endonuclease cleaving of DNA in agarose blocks, separation of the DNA fragments by PFGE and double cleavage techniques are done as reported[17,18]. Digestion by I-*Ceu*I, including partial digestion, uses the methods described earlier[11]. For partial digestion, the enzyme concentration must be diluted; it is important to titer the enzyme prior to use.

Tn*10* insertions

The transposon Tn*10* has one site for the endonuclease *Xba*I, and two sites for *Bln*I (=*Avr*II); thus insertions of Tn*10* into specific genes enables the mapping of these genes on the physical chromosome. Many genes which contained Tn*10* insertions were mapped on the chromosome of *S. typhimurium* LT2[17,19]; these transposons were transduced by bacteriophage P22 into related species such as *S. enteritidis*[20], *S.paratyphi* A[21], *S. paratyphi* B[22], and *S typhi*[23], thus permitting the mapping of the location of specific genes by physical analysis of the location of the sites for *Xba*I and *Bln*I.

CHANGES IN THE GENOME

Changes may be of three different types: point mutations (base pair changes); genetic rearrangements (deletions, duplications, translocations, and inversions); additive genetic changes (lateral transfer due to genetic exchange resulting in insertion of non-homologous DNA), resulting in "pathogenicity islands".

Base pair changes

These are detectable by PFGE methods only if they occur in the target site of the digesting enzyme, where they result in restriction fragment length polymorphisms (RFLPs) which can be detected by most endonucleases, and can give an approximate measure of the number of base pair changes. However, the 19 bp site of I-*Ceu*I within the 23S rRNA gene was never lost due to mutation, and was not formed by chance at any site outside the rRNA genes (though other base pair changes occur, and are detected by nucleotide sequencing, or by other endonucleases).

Genetic rearrangements

In most genera and species, these are rarely detected in wild type strains of the enteric bacteria; this is best illustrated by the fact that the chromosomes of *S. typhimurium* LT2 and *E. coli* K-12 have retained similarity of genes and gene order[1,2]. However, two types of genetic changes are detected, at least in some species.

rrn-operon exchanges

Based on genomic maps for the enzymes *Xba*I, *Bln*I, *Spe*I, and I-*Ceu*I, rearrangements due to inversions and translocations resulting from homologous recombination between the *rrn* operons are very rare in most species; the I-*Ceu*I fragments are in the order ABCDEFG in each of the species *S. typhimurium*[18] (Figure 1), *S. paratyphi* B[22], and *S. enteritidis*[20], the same order as in *E. coli* K-12[15]. Because I-*Ceu*I cleaves only *rrn* operons and because the *rrn* skeleton is highly conserved in enteric bacteria, related wild type strains usually yield identical fingerprints; for example, seventeen independent wild type *S. typhimurium* strains gave very similar I-*Ceu*I digests, indicating conservation of the order of I-*Ceu*I fragments on the chomosome[11].

However, in some of the species of *Salmonella*, especially those which are host-specialized, the order of I-*Ceu*I fragments is rearranged, although the order of genes on individual fragments is similar[23,24]. These rearrangements result from homologous recombination between pairs of the seven *rrn* operons, which are repetitive DNA in a chromosome in which most of the DNA is unique. For example, the order of I-*Ceu*I fragments in *S. typhi* Ty2 (limited to growth in humans) is changed to AGCEFDB (Figure 2). The *rrn* skeletons of 127 wild type strains of *S. typhi* showed 21 different orders, which we called "genome types", postulated to be due to inversions and translocations with *rrn* endpoints[25] (Figure 3). The genomes of strains of *S. paratyphi* A[21] and C[26] (both also limited to growth in humans) are similarly rearranged, as are the genomes of *S. gallinarum* and *S. pullorum*

(limited to growth in fowl) (Liu and Sanderson, unpublished data). The order of I-*Ceu*I fragments in the chromosomes of other species of *Salmonella*, most of which are host-generalists able to grow in many hosts, is strongly conserved (Liu and Sanderson, unpublished data). Postulated patterns of homologous recombination between the seven *rrn* operons are shown in Figure 4.

Other enteric bacteria also show some rearrangements. The order of genes on the chromosome of *Klebsiella oxytoca* M51a resembles *S. typhimurium*, but the chromosome differs as follows: there are eight *rrn* operons instead of seven; one of the I-*Ceu*I fragments is translocated to a new location; the total size of the chromosome is 5200 kb rather than 4800 kb as in *S. typhimurium* (Figure 1)[27]. Fourty other wild type strains of *Klebsiella* spp. all have eight *rrn* operons, and in a few cases there are further translocations of the I-*Ceu*I fragments (Liu and Sanderson, unpublished data).

Inversions in TER region

The second category of genetic rearrangement involves inversions over TER, the terminus region of the chromosome. This is the only region in which inversions in wild type strains of *Salmonella* are common (other than inversions involving *rrn* genes, discussed above). Inversions overlap the TER region in the following species, when compared with *S. typhimurium* (the size of the inversion in kb is shown): *E. coli*, 480 kb[15,16]; *S. enteritidis*, 750 kb[20];*S. typhi* , 500 kb; *S. paratyphi* C, 700 kb[26]. No inversion was detected in *S. paratyphi* B[22]. All these inversions differ by at least one end-point. The gene order in *S. typhimurium* may be considered the ancestral order, because all inverted orders could be obtained in single events from the *S. typhimurium* order, whereas more complex events would be required from other orders, but this may simply reflect high frequency of inversions in this region. Inversions at many points in the genome are detected in experimental studies in *E. coli* K-12 and *S. typhimurium* LT2, though some endpoints are much less common than others[7,28]. Two types of recombination occur at high frequency in *E. coli* K-12 in the TER region: *recA*-dependent homologous recombination, resulting in deletions[29]; site-specific recombination at the *dif*-site in the TER region, due to activity of two related recombinases XerC and XerD of the lambda integrase family[30]. It is likely that these types of recombination are responsible for inversion in wild type strains in *Salmonella* species, though this is not yet proven.

Addition of new genes through lateral transfer

This involves entry of non-homologous genes, presumably by classical genetic transfer methods such as conjugation and transduction, and integration of these genes into the chromosome. This results in segments or "loops" of DNA in one strain for which there is no homologue in closely related species or genera. Loops of DNA distinguishing *E. coli* and *S. typhimurium* were noted by Riley and colleagues[2,31]. These loops have been called pathogenicity islands (though not all such loops may function in pathogenicity); in many cases they include a block of genes contributing to a specific virulence phenotype. Two *Salmonella* pathogenicity islands (SPI), both of about 40 kb, have been identified; SPI-1 governs the ability of *Salmonella* to invade epithelial cells[32] and SPI-2 mediates survival within macrophages[33,34]. These pathogenicity islands distinguish different genera of enteric bacteria, but chromosomes of different species of *Salmonella* also differ by loops of this type, and PFGE analysis has identified these loops by two methods. Firstly, genetic maps of *Salmonella* show that intervals between the same gene pair in different species may be very different. For example, in *S. typhi* the *mel-poxA* interval is 120 kb longer than in *S. typhimurium* and other species; this loop was found to carry the *viaB* gene for the Vi antigen, missing from most other *Salmonella*[23,25]. This suggested the existence of a pathogenicity island; this was confirmed, for a site-specific recombinase[35] and the synthesis of type IV pili[36] were shown to be controlled by genes in this major pathogenicity island of *S. typhi*. Secondly, changes in the length of the I-*Ceu*I fragments indicates insertions or deletions; e.g. the I-*Ceu*I-D fragment is 136 kb in *S. typhi* but only 92 kb in all other *Salmonella* species examined so far, indicating an insertion of about 44 kb in this region[23].

Pathogenicity islands have also been identified in *E. coli* strains of the enteropathogenic *E. coli* (EPEC) group and the uropathogenic *E. coli* group (UPEC), as well as in *Yersinia pestis* and *Vibrio cholerae*[37]. Lateral transfer resulting in "loops" of species-specific DNA is a major genetic mechanism resulting in species adaptation in the enteric bacteria, including *Salmonella*. These islands of unique DNA appear to be responsible for the major differences in pathogenicity between the closely related species and genera of the enteric bacteria. In addition, considerable genome size variation was detected among recent isolates of *S. typhi*, based on PFGE[39].

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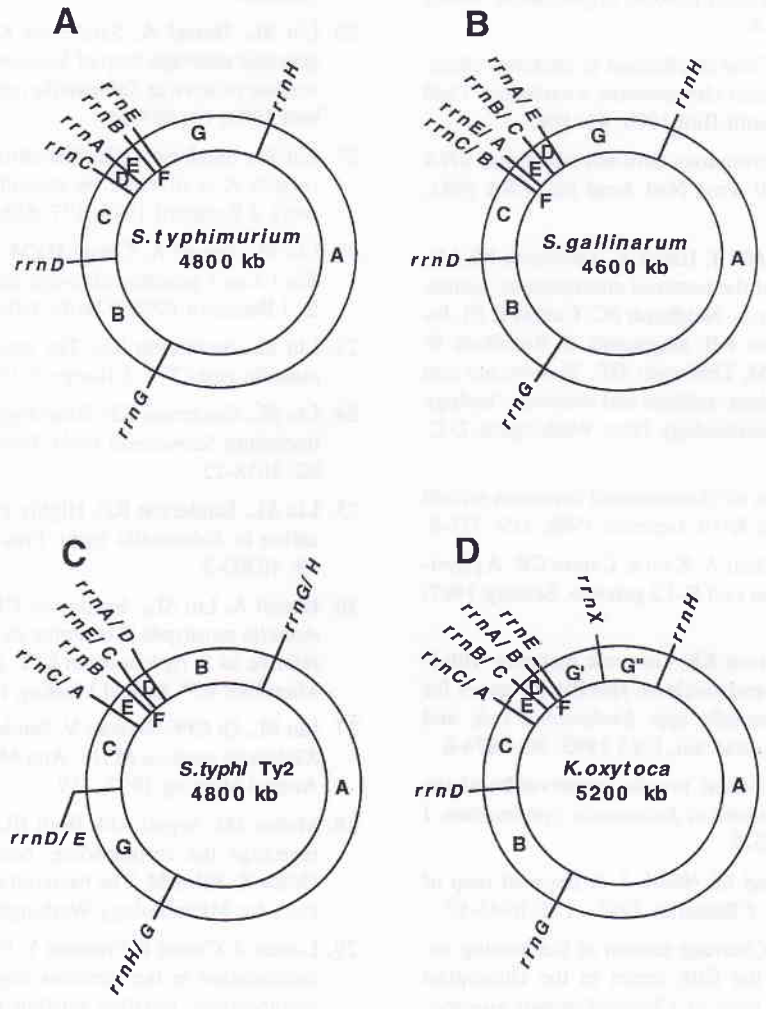

Figure 1. *The "rrn genomic skeleton" of the chromosomes of S. typhimurium LT2 (A), as reported earlier[18], of S. gallinarum (B) (unpublished data), of S. typhi Ty2 (C)[23-25], and of Klebsiella oxytoca (D) (Liu SL and Sanderson KE, unpublished data) The letters A to G indicate the map order of fragments obtained by digestion with the endonuclease I-CeuI. The rrn operon designation at the site of I-CeuI digestion is based on detailed genetic data reported earlier[18] for S. typhimurium ; for other species hybrid rrn operons are named according to postulated homologous recombination between rrn operons, or are named according to location. Each of the species has seven rrn operon except for K. oxytoca, which also has an eighth rrn operon designated rrnX.*

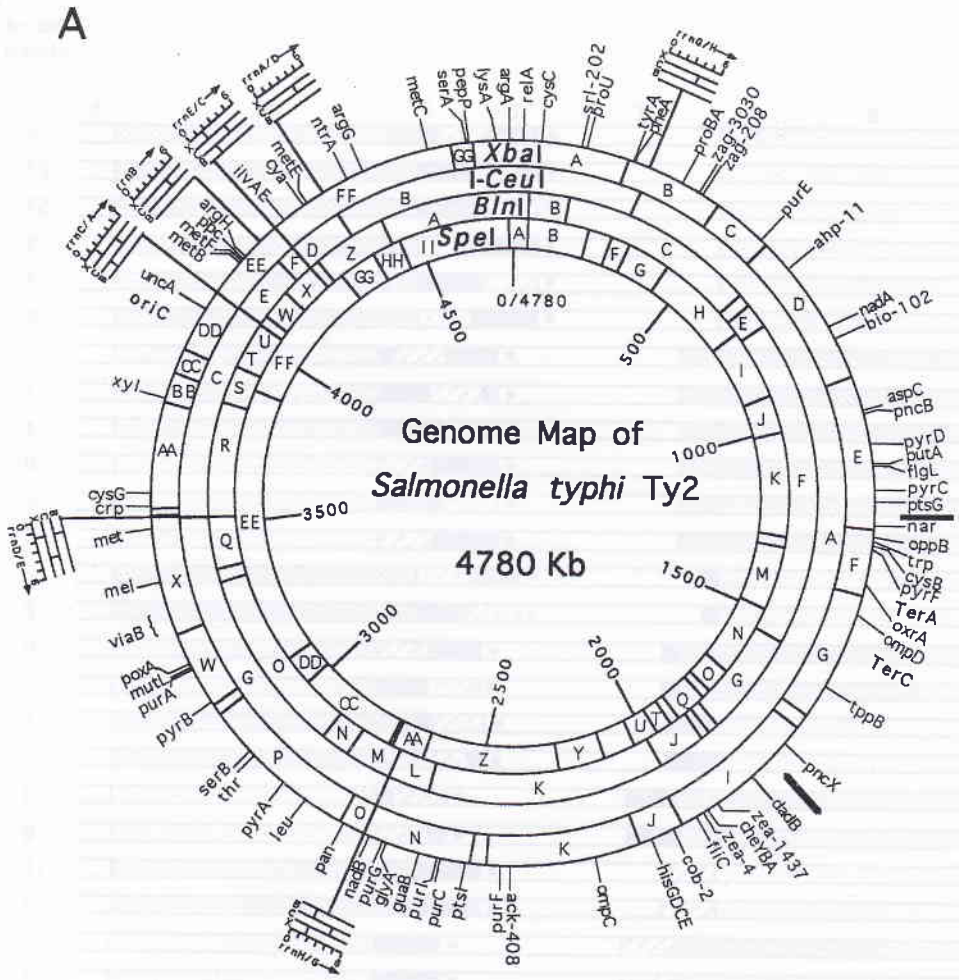

Figure 2 A. *Genomic cleavage map of S. typhi Ty2 for XbaI, I-CeuI, BlnI, and SpeI. The size of the map in kbs is on the inside of the circle. For most enzymes the fragments are named in alphabetical order, beginning with A at 0 kb; for I-CeuI, the fragments are named to be homologous with those of other Salmonella spp. and E. coli, but the order of I-CeuI fragments in S. typhi is rearranged to AG-CEFDB[24]. The enzyme I-CeuI digests the seven rrn operons in the rrl gene for 23S-rRNA[13]. The structure of the rrn operons is shown in detail outside the circle; the positions of the cleavage sites in the rrn genes are from double digestion data. The four rrn genes which have XbaI and BlnI sites are inferred to have the tRNA gene for glutamate-tRNA, and the other three to have the gene for alanyl-tRNA, by analogy with E. coli[38]. The arrow indicates the direction of transcription originally determined for E. coli in rrnB[38]. The relative locations of endonuclease cleavage sites were determined by double digestion data (see text). The genes are located through mapping the XbaI and BlnI sites in Tn10 insertions in the genes. The region which is inverted with respect to S. typhimurium is marked by bars at about 1200 kb and 1800 kb.*

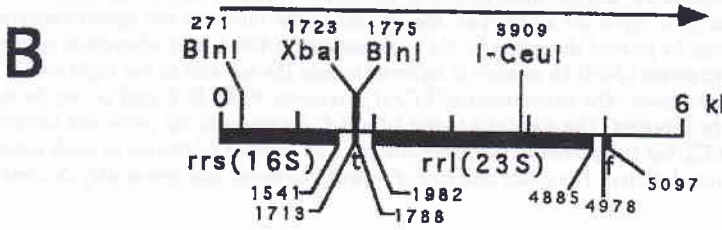

Figure 2 B. *Structure of the rrnB operon of E. coli[38]; endonuclease cleavage sites were determined from nucleotide sequence, and the order of the genes is rrs (16S rRNA)-tRNA-rrl (23S-rRNA)-rrf (5S rRNA). The nucleotide number of cleavage sites and of ends of genes are shown.*

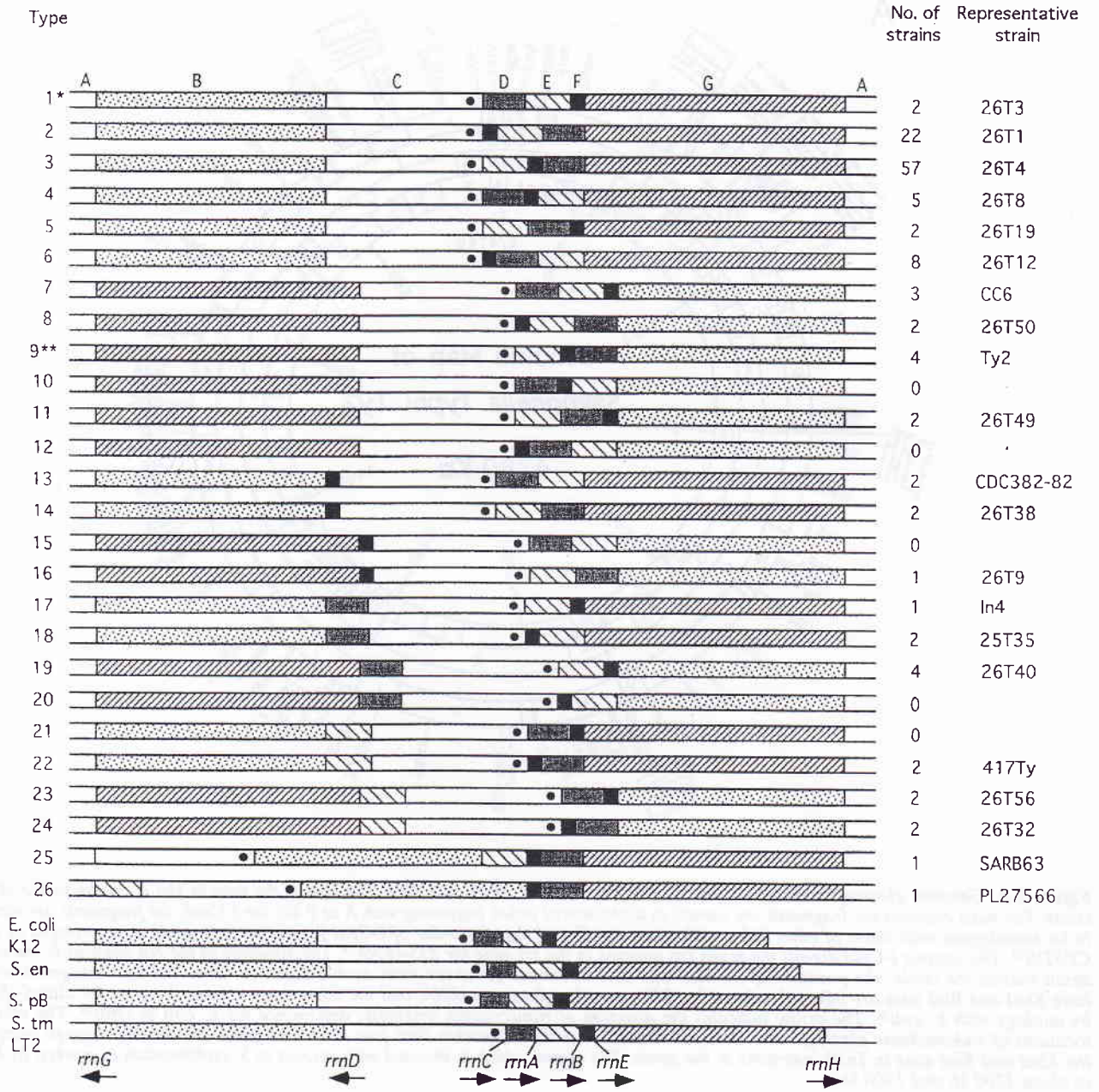

Figure 3. *The order of I-CeuI fragments in 126 strains of S. typhi. The order in four species which show very little variability within each species is also shown, E. coli K-12, S. enteritidis (E.en), S. paratyphi B (S.pb), and S. typhimurium LT2 (S.tmLT2). The sizes in kb of the fragments in most strains of S. typhi are at the top; the lengths of the intervals are approximately to scale. The order of I-CeuI fragments B to G was determined by partial digestion by the endonuclease I-CeuI, and separation of the fragments by pulsed-field gel electrophoresis. The I-CeuI-A fragment (2400 kb in size) is inferred to join the left end to the right end of the fragments shown, forming a circle, but its orientation is not known. The orientation of I-CeuI fragments B, D, E, F, and G can be inferred from the polarity of the rrn genes, but I-CeuI-C could be inverted. The solid dot in the I-CeuI-C fragment is the proposed location of oriC; this is known in E. coli K-12 and S. typhimurium LT2, but is inferred in the other strains. The number of strains of each genome type is shown; some of the genome types shown here were not detected. The order and size of I-CeuI fragments was previously determined in S. typhimurium LT2*[18,23].

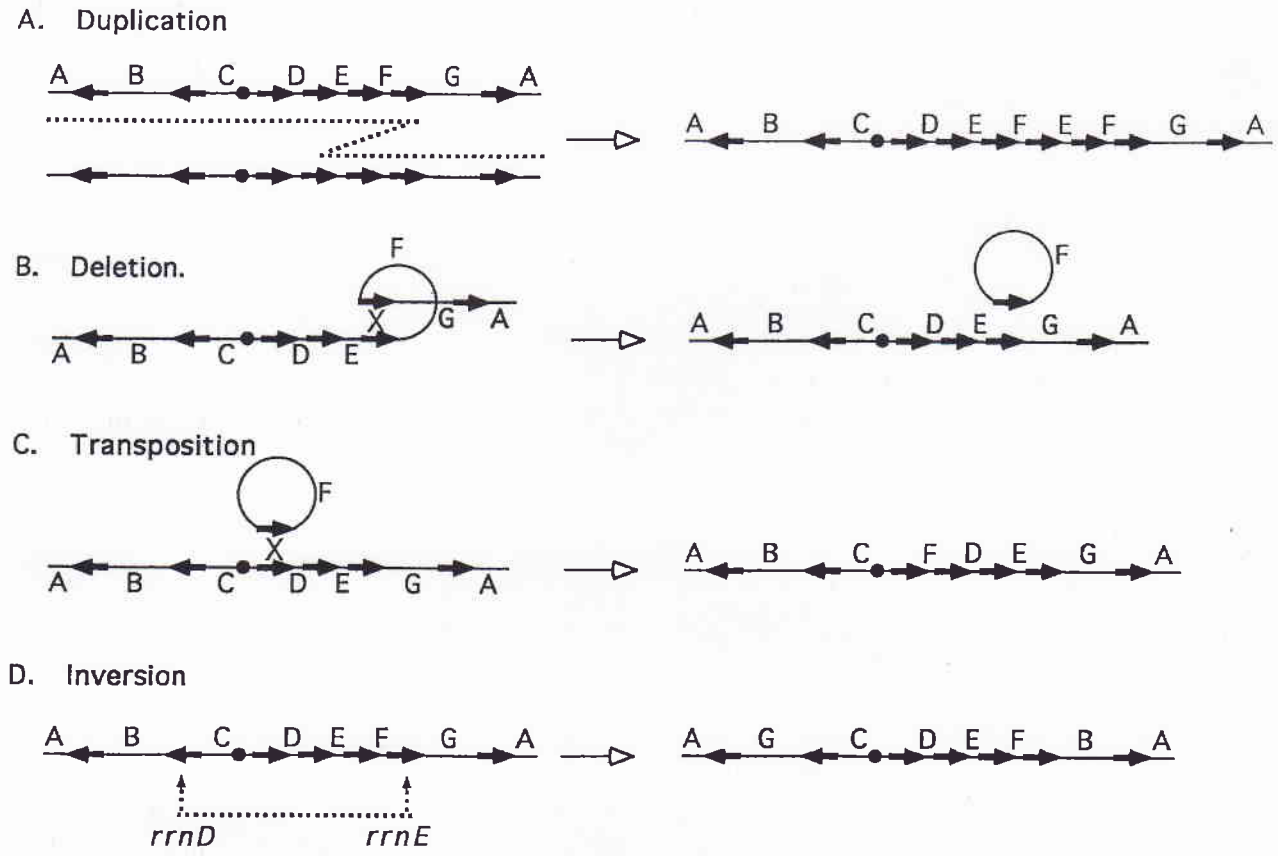

Figure 4. *Postulated patterns of homologous recombination between the rrn operons. The arrows show the rrn operons and the orientation of their transcription. The letters refer to the I-CeuI fragments between the rrn operons. In each case the initial order is type 1 in Figure 3, as found in S. typhimurium LT2. a) and b). Duplications and deletions can occur by intrachromosomal recombination. c) Transposition results if the deleted segment (as in "b") re-inserts into the chromosome at a different rrn operon by homologous recombination. d) Inversion results from recombination between genes which are oriented in different directions, as shown here in the dotted line between rrnD and rrnE. This rearrangement results in types 7 to12 (Figure 3).*